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Supplemental Information

Conditionally Stabilized dCas9 Activator for Controlling Gene Expression in Human Cell Reprogramming and Differentiation

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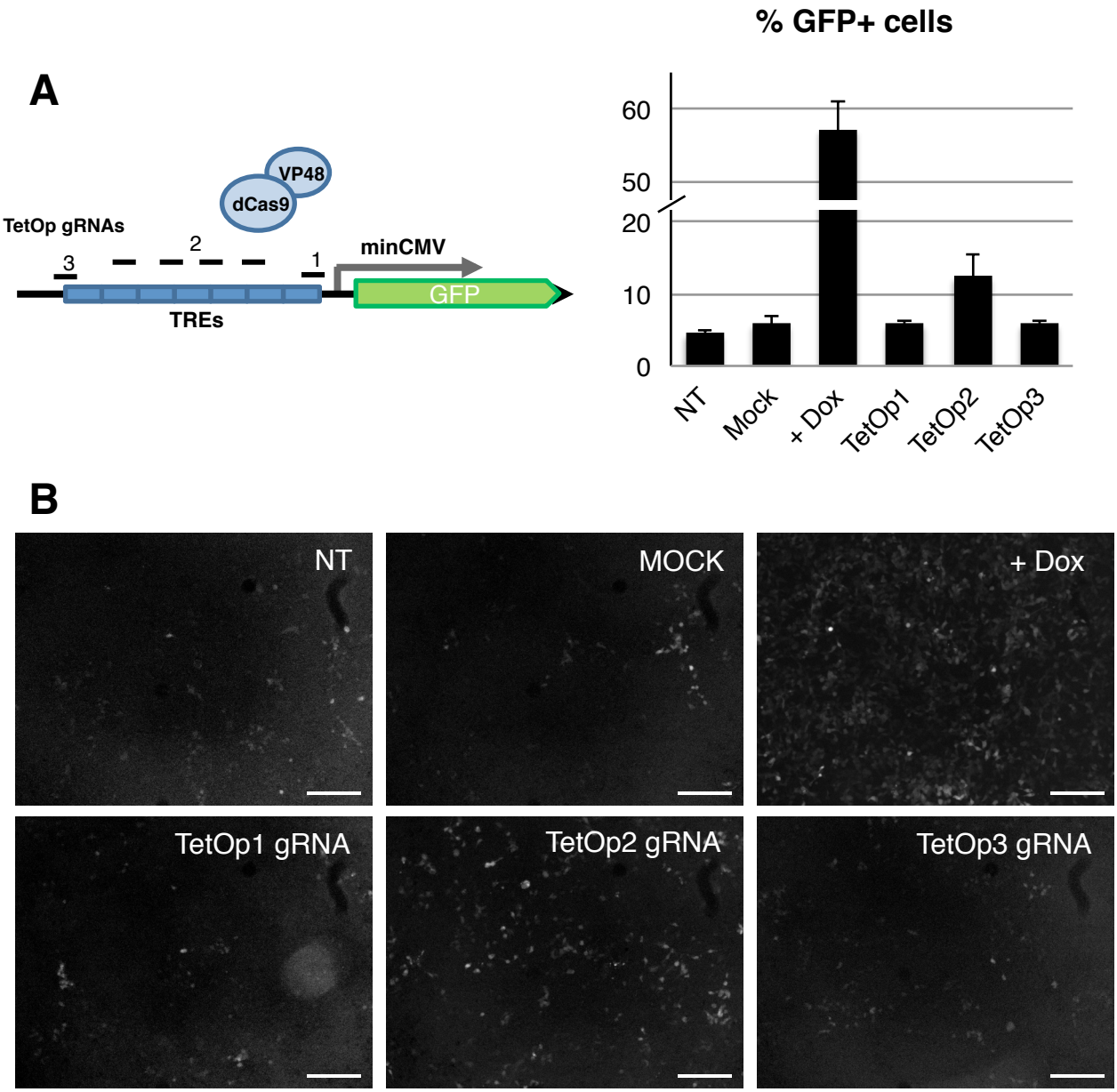

Figure S1. Validation of dCas9VP48 activator. Related to Figure 1.

(A) dCas9VP48 construct was transfected together with gRNAs targeting the tetracycline response element (TRE) repeats in the Tet-ON promoter. Number of GFP+ HEK293 cells was analyzed by flow cytometry. Data represent mean ± SEM, n = 3 independent transfections. Addition of doxycycline (+Dox) to activate rtTA-mediated GFP-expression was used as a positive control and non-transfected (NT) and dCas9VP48-only transfected (Mock) were used as negative controls.
(B) Representative fluorescence microscopy fields of GFP+ cells in the different conditions. Scale bars 200 μm.

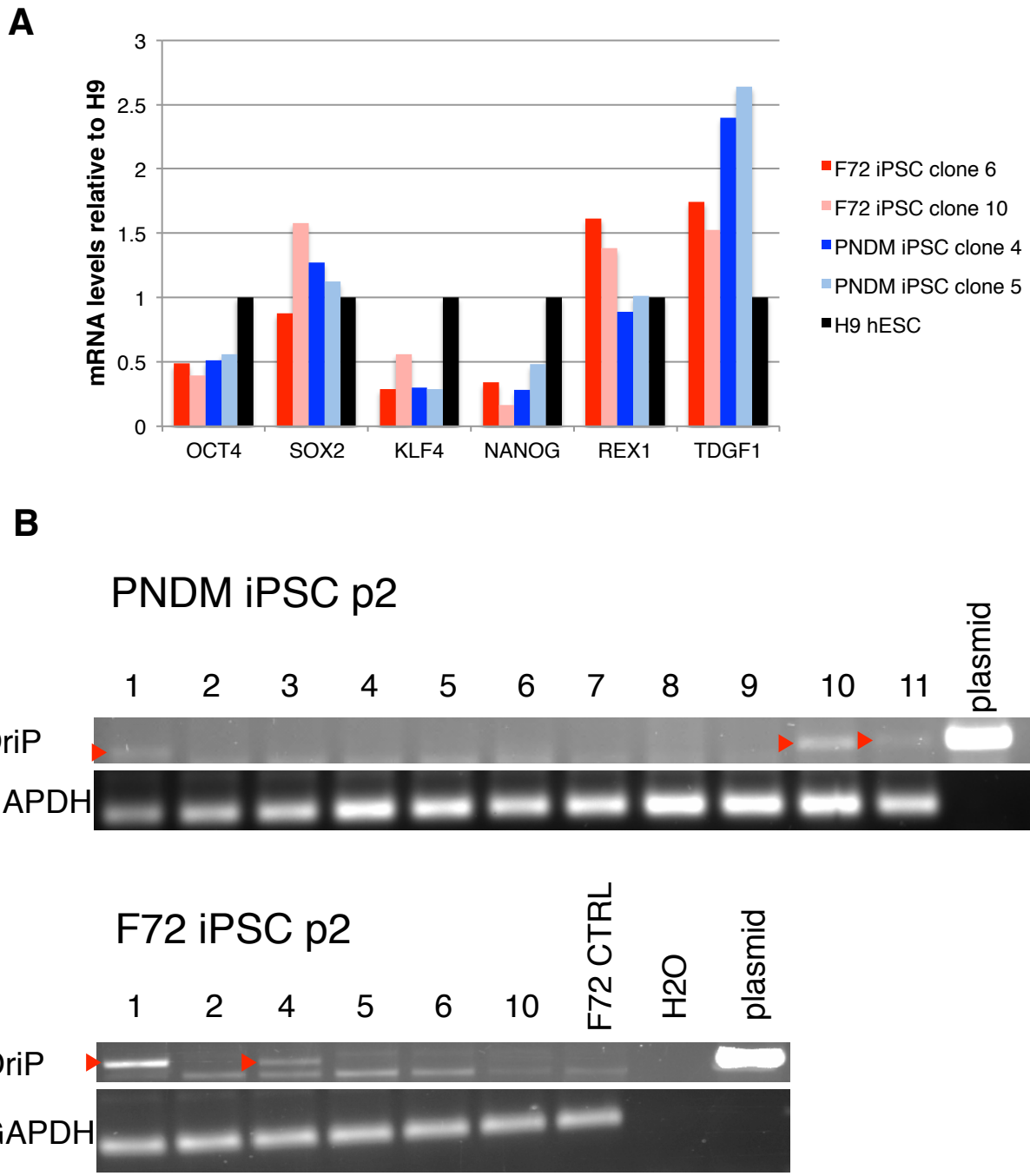

Figure S2. Characterization of dCasVP192 OCT4 reprogrammed iPSC. Related to Figure 2.

(A) qRT-PCR characterization of reprogrammed iPSC clones. mRNA levels of pluripotency markers relative to H9.
(B) Episome detection PCR of DNA preparations from dCasVP192 OCT4 reprogrammed iPSC. Red arrows indicate persistent or integrated plasmids in assayed clones.

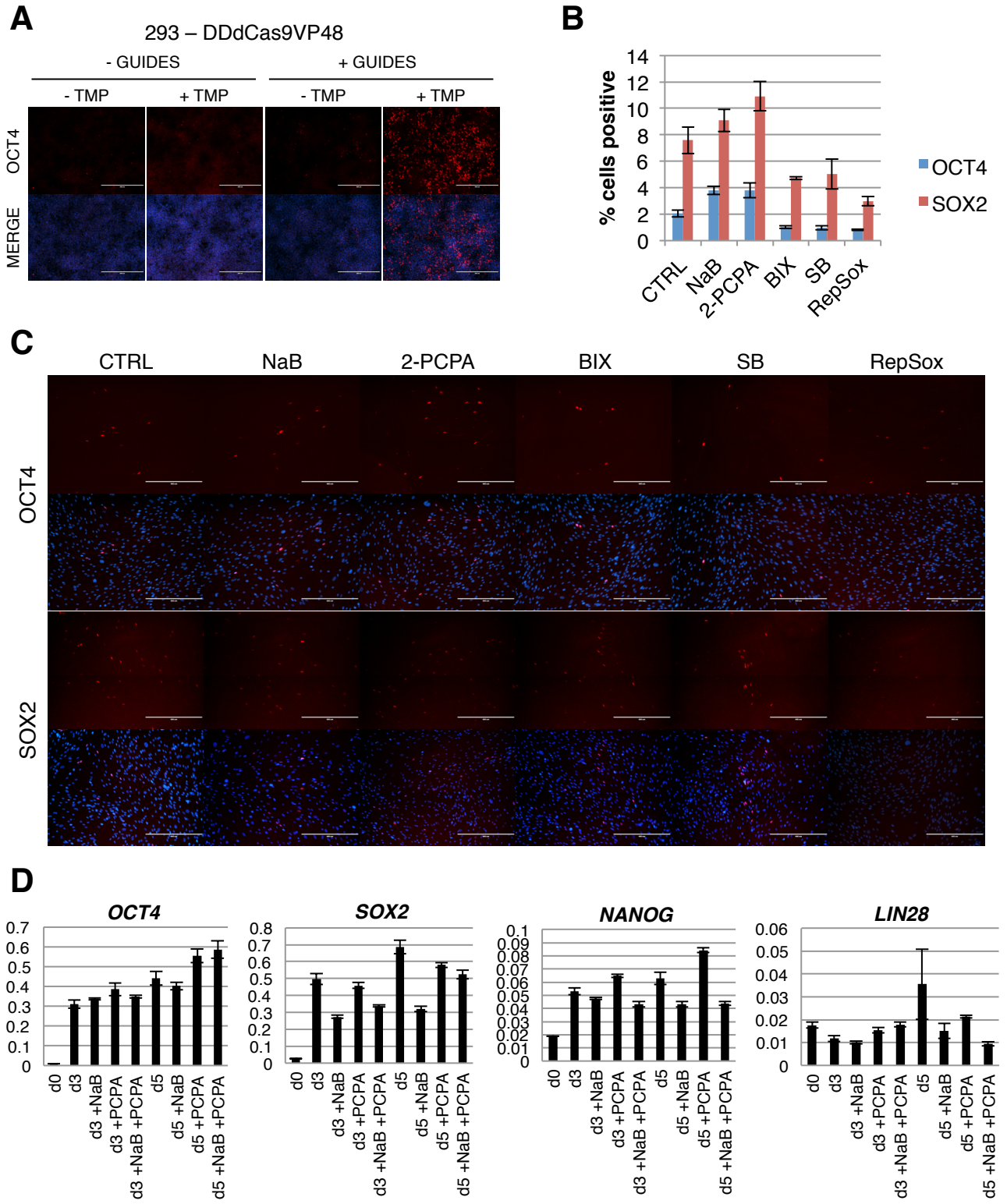

Figure S3. Effect of small molecular compounds on human fibroblast gene activation. Related to Figure 3.

(A) TMP dependent *OCT4* activation in HEK293 cells expressing DDdCas9VP48 and OCT4 targeting guides. Scale bars = 400 μm in A and C.

(B)(C) Number of OCT4 and SOX2 positive cells counted per visual field and representative immunocytochemical stainings of F72 fibroblasts expressing DDdCas9VP192 and *OCT4*, *SOX2*, *NANOG* and *LIN28A* targeting guides and treated with TMP and inhibitors. NaB = sodium butyrate, 2-PCPA = *trans*-2-Phenylcyclopropylamine (Tranylcypromine), BIX = BIX01294, SB = SB431542.

(D) Activation of *OCT4, SOX2 NANOG* and *LIN28A* by qPCR in the presence of 250 μM NaB and 4 μM PCPA after 3 and 5 days of 1 μM TMP treatment in F72 expressing DDdCas9VP192 and OCT4, SOX2, NANOG and LIN28A targeting guides. Data represented as mean ±SEM, n=3.

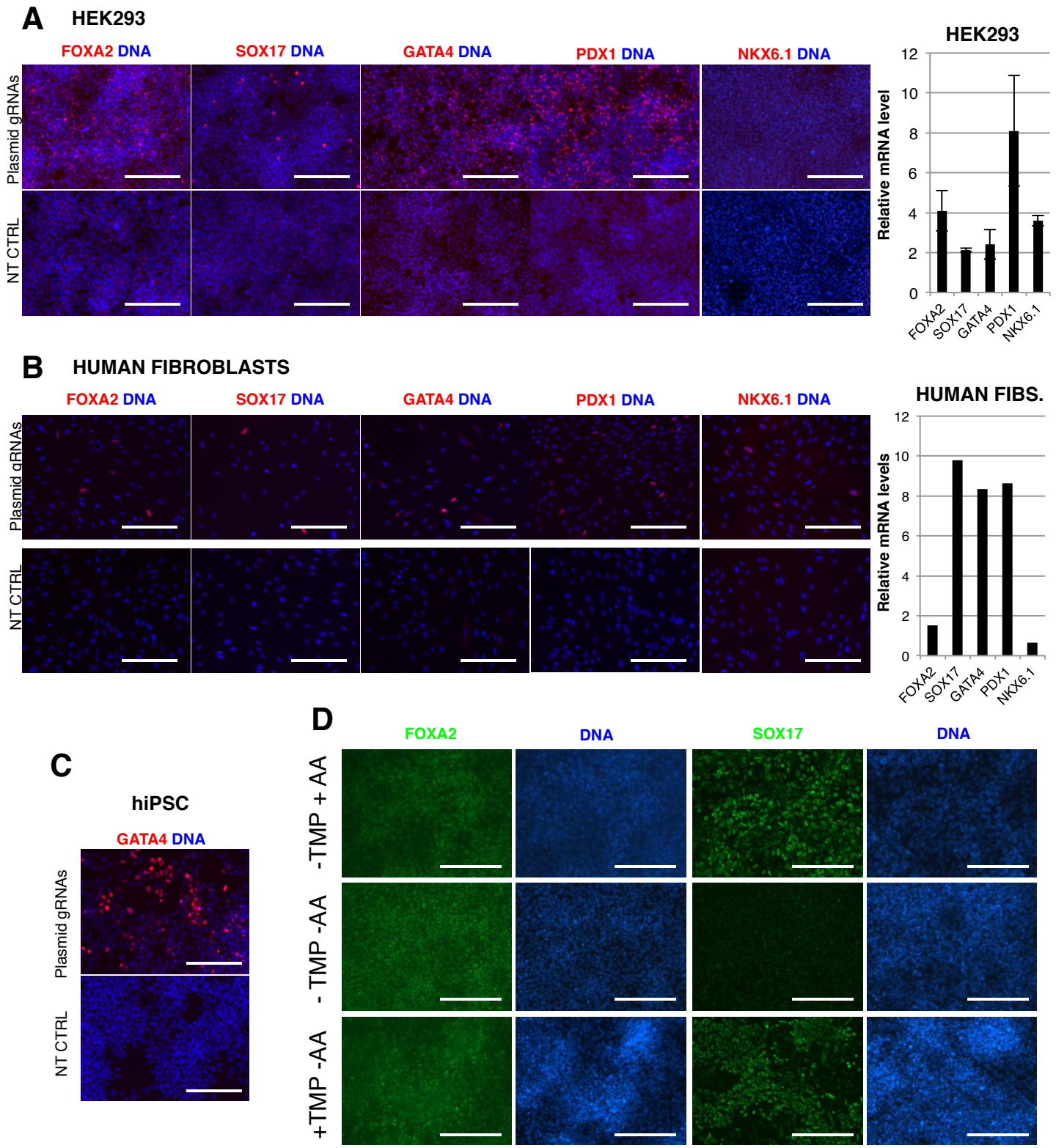

Figure S4. Activation of endodermal lineage specific transcription factors by dCas9VP192 mediated transcriptional activation. Related to Figure 4.

(A)(B) Immunocytochemical detection and qPCR analyses of indicated targeted gene products in HEK293 and F72 human foreskin fibroblasts. Analysis was performed 72 h after delivery of gRNA-concatenated plasmids and dCas9VP192. mRNA levels relative to non-treated cells, represented as mean ± SD, n=2 for HEK293 and n=1 for F72. Scale bars = 200 μm for all panels.
(E) Immunocytochemical detection of GATA4 in hiPSC transfected with dCas9VP192 and gRNAS.
(D) Immunocytochemical detection of FOXA2 and SOX17 in hESC expressing DDdCas9VP192 and gRNAs for FOXA2, SOX17, PDX1 and NKX6.1 differentiated to definitive endoderm at day 3.

Supplemental Experimental Procedures

Plasmid construction

Catalytically inactive dCas9 was cloned by introducing H840A mutation by PCR into pX334-U6-DR-BB-DR-Cbh-NLS-hSpCas9n(D10A)-NLS-H1-shorttracr-PGK-puro (pX334) (Addgene plasmid: 42333) (Cong et al., 2013). The dCas9 construct was cloned into pX335-U6-Chimeric_BB-CBh-hSpCas9n(D10A) (Addgene plasmid: 42335) along with PKG Puro from pX334 to create pX335-U6-Chimeric_BB-CBh-dCas9-PGK-Puro. VP16 transactivation domain repeats were cloned from rtTA by PCR and fused C-terminally to dCas9 in pX335-U6-Chimeric_BB-CBh-dCas9-PGK-Puro to create dCas9 transactivator constructs. T2A-GFP was fused C-terminally to dCas9VP192 and cloned into CAG-IRES-Puro backbone to create CAG-dCas9VP192-GFP-Puro construct. For episomal replicating plasmids dCas9VP192-GFP fragment was cloned from CAG-dCas9VP192-GFP-Puro into pCXLE backbone (pCXLE-hSK; Addgene plasmid: 27078; Okita et al., 2011) with EcoRI. pCXLE-dCas9VP192-GFP-shp53 was cloned by inserting dCas9VP192-GFP into backbone from pCXLE-hOCT3/4-shp53-F (Addgene plasmid: 27077). pCXLE-hOCT4 was cloned form pCXLE-hOCT3/4-shp53 by removing the p53 shRNA sequence between BamHI sites. PiggyBac constructs encoding the dCas9VP192-GFP-IRES-Neo were cloned by inserting the CAG dCas9 activator between PiggyBac recombination sites in pPB-R1R2-NeoPheS (Yusa et al., Nature, 2011). Destabilized dCas9 activator was generated by PCR cloning the DHFR(DD) from pBMN DHFR(DD)-YFP (Addgene plasmid: 29352) and inserting it N-terminally in fusion with the dCas9 in the PB-CAG-dCas9VP192-GFP-IRES-Neo. MAFA overexpression construct was generated by PCR cloning the human MAFA cDNA into PB-tight-ires-EmGFP backbone.

Guide RNA production

Guide RNA transcriptional units (gRNA-PCR) were prepared by PCR amplification with Phusion polymerase (ThermoFisher), using as template U6 promoter and terminator PCR products amplified from pX335 together with a guide RNA sequence-containing oligo to bridge the gap. PCR reaction contained 50 pmol forward (Fw) and reverse (Rev) primers, 2 pmol guide oligo, 5 ng U6 promoter and 5 ng terminator PCR products in a total reaction volume of 100μl. PCR reaction program was 98C/10sec, 56C/30sec, 72C/12sec for 35 cycles. Amplified gRNA-PCRs were purified and confirmed by sequencing. When needed, alternative Fw and Rev primers were used to incorporate suitable restriction sites for gRNA-PCR concatenation.

gRNA-PCR units were concatenated using Golden Gate assembly (Cermak et al., 2011) . Destination vector GGdest-ready was generated by PCR-cloning Esp3I destination cassette from pCAG-T7-TALEN(Sangamo)-Destination (Addgene plasmid: 37184; (Hermann et al., 2014) into pGEM-4Z (Promega). Assembly reactions contained 150 ng of GGdest-ready vector, 50 ng of each gRNA-PCR product (five in total), 1 uL Esp3I (Thermo Fisher, ER0451), 2 uL T4 DNA ligase (Thermo Fisher, EL0011), 2 uL T4 ligase buffer and 2 uL DTT (10mM, Promega, V3151) in a final volume of 20 uL. Thermal cycle consisted of 50 cycles of restriction/ligation (2 min at 37°C, 5 min at 16°C) followed by enzyme inactivation step (20 min at 80ºC). Ten microliters of the reaction were transformed into DH5alpha chemical competent bacteria and plated on LB agar containing ampicillin with IPTG/X-Gal for blue/white recombinant screening. Correct concatenation of the gRNA-PCR products was confirmed by sequencing.

gRNAs concatenated into the GGdest-ready were further cloned into either EBNA or PiggyBac backbones for the different experiments.

Cell transfection

HEK293 cells were seeded on tissue culture treated 24 well plates one day prior to transfection (10^5 cells/well). hESC and hiPSC cells were split to Matrigel-coated 24-well plates 48 hours before transfection. Cells were transfected using FuGENE HD transfection reagent (Promega) in fibroblast culture medium or E8 stem cell media with 500 ng of dCas9 transactivator encoding plasmid and 100 to 200 ng of gRNA-PCR or 500 ng gRNA-PCR. Cells were cultured for 72 hours post-transfection, after which samples were collected for FACS analysis, qRT-PCR or immunocytochemical staining.

Cell electroporation

Human skin fibroblasts were electroporated using Neon Transfection system (Life Technologies) according to manufacturer's instructions. Briefly, 1 million cells were electroporated in 100 μl tips with 1650 V, 10 ms and 3x pulse settings. Amounts of DNA used were: 6 μg of total plasmid consisting of 3 μg of guide template EBNA plasmids and 3 μg of pCXLE-dCas9VP192-GFP-shp53 per electroporation. For OCT4 replacement experiments 2μg of pCXLE-dCas9VP192-GFP-shp53, 2μg of OCT4 gRNA plasmid, 1μg of pCXLE-hSK and 1μg of pCXLE-hUL were used. For transgenic OCT4 control OCT4 gRNA plasmid was replaced with pCXLE-hOCT3/4-shp53 (2μg) and dCas9VP192 plasmid was replaced with pCXLE-GFP (2μg). For selected inducible cell lines fibroblasts were electroporated with 1μg of PB-transposase, 2μg of PB-CAG-DDdCas9VP192-GFP-IRES-Neo, 1.5μ of PB-OCT4-SOX2-guides-PGK-Puro and 1.5μg of PB-NANOG-LIN28A-guides-

PGK-Puro plasmids. After electroporation cells were plated in fibroblast medium on gelatin-coated tissue culture plates and selected with G418 (Roche) and puromycin (Life Technologies).

For the generation of the hESC line used in the differentiation experiments, 2 million H9 (WiCell) cells were electroporated with 2 μg of PB-CAG-DDdCas9VP192-GFP-IRES-Neo, 1 μg PB-FOXA2-SOX17-guides-PGK-Puro, 1 μg PB-PDX1-NKX6.1-guides-PGK-Puro and 1μg of PB-transposase using Neon Transfection system (1100 V, 20 ms, 2x pulses). Cells were plated onto a Matrigel coated plate with E8 medium containing 5 uM ROCK inhibitor (Y-27632 2HCl, Selleckchem). 72 h after electroporation and recovery cells were selected with G418 and puromycin.

hESC differentiation to endoderm and pancreatic lineage

hESC expansion and plating, and media used for differentiation to definitive endoderm was performed as described in Rezania et al., 2014. Briefly, confluent plates of H9 hESC cells were washed twice with PBS and incubated for 72 h in the following endoderm differentiation media: d0: MCDB131 (Life Technologies) + 2mM Glutmax (Life Technologies) + 1.5 g/L NaHCO3 (Sigma) + 0.5% BSA fV (Sigma) + 10mM final Glucose (Sigma) + 100 ng/ml Activin A (Dr Marko Hyvonen, Department of Biochemistry, University of Cambridge) + 3 μM CHIR; d1: Identical to d0 but only 0.3 μM CHIR; d2: Identical to d0 but no CHIR. After definitive endoderm stage, cells were differentiated to pancreatic progenitors as described in Rezania et al., 2014 .

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde (PFA), permeabilized using 0.2% Triton-x100 and blocked using Ultra V Block (Thermo Fisher). Primary antibodies were diluted in PBS

containing 0.1% Tween-20 and incubated overnight in +6°C. Secondary antibody incubations were done in room temperature for half an hour. After antibody incubations, cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) or Hoechst and cells were imaged under fluorescent microscope (EVOS, Life Technologies). Primary antibodies used were: OCT4 (1:500, C30A3, Cell Signaling; 1:100, MA1-104, Thermo; 1:500, SC-8628, Santa Cruz), SOX2 (1:500, D6D9, Cell Signaling), LIN28A (1:500, D84C11, Cell Signaling; 1:100, MA1-016, Thermo), NANOG (1:500, D73G4, Cell Signaling; 1:100, MA1-017, Thermo), E-cadherin (1:500, 610181, BD Bioscience), KLF4 (1:250, HPA002926, Sigma-Aldrich), FOXA2 (1:500, SC-9187, Santa Cruz), PDX1 (1:200, AF2419, R&D Systems), NKX6.1 (1:200, F55A10, Developmental Studies Hybridoma Bank), SOX17 (1:500, AF1924, R&D Systems), GATA4 (1:500, SC-1237, Santa Cruz) and MAFA (1:500, AB26405, Abcam). Secondary antibodies used were AlexaFluor 488/594: donkey anti-goat (1:500, A11055 and A11058; Invitrogen), donkey anti-mouse (1:500, A21202 and A21203; Invitrogen) and donkey anti-rabbit (1:500, A21206 and A21207; Invitrogen). For estimation of activation efficiency from immunostainings cells were manually counted from four random fields per sample.

Flow cytometry

For GFP+ flow cytometry, cells were dissociated using incubation with TrypLE Select (Life Technologies 12563029) for 5 min at 37ºC, counted and resuspended in FACS-buffer (5% FBS in PBS). Samples were run with FACSCalibur (BD Biosciences) and analyzed with CellQuest-Pro software (BD Biosciences). Non-treated cells were used as control for gating.

Quantitative RT-PCR (qRT-PCR) analysis

Gene expression was assessed using SYBR-Green based qRT-PCR, performed as previously described elsewhere (Toivonen et al., 2013). Relative expression was analysed using ΔΔCt method, with cyclophilin G (*PPIG*) as endogenous control and an exogenous positive control used as calibrator. Expression levels are relative to non-treated cells or to hESC as indicated in the figure legends.

Primers for qRT-PCR

Gene	Reference	Forward	Reverse	Product size (bp)
CYCLOG	NM_004792	TCTTGTCAATGGCCAACAGAG	GCCCATCTAAATGAGGAGTTG	84
OCT4	NM_002701	TTGGGCTCGAGAAGGATGTG	TCCTCTCGTTGTGCATAGTCG	91
SOX2	NM_003106	GCCCTGCAGTACAACTCCAT	TGCCCTGCTGCGAGTAGGA	85
NANOG	NM_024865.2	CTCAGCCTCCAGCAGATGC	TAGATTTCATTCTCTGGTTCTGG	94
LIN28	NM_024674	AGGAGACAGGTGCTACAACTG	TCTTGGGCTGGGGTGGCAG	74
KLF4	NM_004235.4	CCGCTCCATTACCAAG	CACGATCGTCTTCCCCTCTT	80
CDH1	NM_004360	ATGAGTGTCCCCCGGTATCT	GGTCAGTATCAGCCGCTTTC	91
FOXA2	NM_021784	AAGACCTACAGGCGCAGCT	CATCTTGTTGGGGCTCTGC	93
SOX17	NM_022454	CCGAGTTGAGCAAGATGCTG	TGCATGTGCTGCACGCGCA	103
GATA4	NM_002052	GAGGAAGGAGCCAGCCTAGCAG	CGGGTCCCCCACTCGTCA	83
PDX1	NM_000209.3	AAGTCTACCAAAGCTCACGCG	CGTAGGCGCCGCCTGC	52
NKX6.1	NM_006168	TATTCGTTGGGGATGACAGAG	TGGCCATCTCGGCAGCGTG	91
NKX2.2	NM_002509	GAACCCCTTCTACGACAGCA	ACCGTGCAGGGAGTACTGAA	82
SOX9	NM_000346	ATCAAGACGGAGCAGCTGAG	GGCTGTAGTGTGGGAGGTTG	100
MAFA	NM_201589	GCCAGGTGGAGCAGCTGAA	CTTCTCGTATTTCTCCTTGTAC	77
GCK	NM_000162	CCGCCAAGAAGGAGAAGGTA	CTTCTGCATCCGTCTCATCA	89

Primers for gRNA amplification and concatenation

U6 promoter fragment PCR	
5pTailedU6promFw	GTAAAACGACGGCCAGTGAGGGCCTATTTCCCATGATTC
U6promRv	GGTGTTTCGTCCTTTCCAC
Terminator fragment PCR	
TermRv80bp	AAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
3pTailedTerm80bpRv	AGGAAACAGCTATGACCATGAAAAAAAGCACCGACTCGGTGCCAC
Term80 Fw	GTTTTAGAGCTAGAAATAGCAAG
Golden Gate concatenation	
1_aggc_Fw	ACTGAATTCGGATCCTCGAGCGTCTCACCCTGTAAAACGACGGCCAGT
1_aggc_Rv	CATGCGGCCGCGTCGACAGATCTCGTCTCACATGAGGAAACAGCTATGACCATG
2_aggc_Fw	ACTGAATTCGGATCCTCGAGCGTCTCACATGGTAAAACGACGGCCAGT
3_aggc_Fw	ACTGAATTCGGATCCTCGAGCGTCTCAGGACGTAAAACGACGGCCAGT
4_aggc_Fw	ACTGAATTCGGATCCTCGAGCGTCTCACCAGGTAAAACGACGGCCAGT
5_aggc_Fw	ACTGAATTCGGATCCTCGAGCGTCTCATGTTGTAAAACGACGGCCAGT
2_aggc_Rv	CATGCGGCCGCGTCGACAGATCTCGTCTCAGTCCAGGAAACAGCTATGACCATG
3_aggc_Rv	CATGCGGCCGCGTCGACAGATCTCGTCTCACTGGAGGAAACAGCTATGACCATG
4_aggc_Rv	CATGCGGCCGCGTCGACAGATCTCGTCTCAAACAAGGAAACAGCTATGACCATG
5_aggc_Rv	CATGCGGCCGCGTCGACAGATCTCGTCTCACGTTAGGAAACAGCTATGACCATG
gRNA oligos	
pOct_1	GTGGAAAGGACGAAACACCGGGGGGAGAAACTGAGGCGAGTTTTAGAGCTAGAAATAG
pOct_2	GTGGAAAGGACGAAACACCGGGTGGTGGCAATGGTGTCTGGTTTTAGAGCTAGAAATAG
pOct_3	GTGGAAAGGACGAAACACCGGACACAACTGGCGCCCCTCCGTTTTAGAGCTAGAAATAG

pOct_4	GTGGAAAGGACGAAACACCGGGCACAGTGCCAGAGGTCTGGTTTTAGAGCTAGAAATAG
pOct_5	GTGGAAAGGACGAAACACCGTCTGTGGGGGACCTGCACTGGTTTTAGAGCTAGAAATAG
pNanog1	GTGGAAAGGACGAAACACCGTCCCAATTTACTGGGATTACGTTTTAGAGCTAGAAATAG
pNanog2	GTGGAAAGGACGAAACACCGTGATTTAAAAGTTGGAAACGGTTTTAGAGCTAGAAATAG
pNanog3	GTGGAAAGGACGAAACACCGTCTAGTTCCCCACCTAGTCTGTTTTAGAGCTAGAAATAG
pNanog4	GTGGAAAGGACGAAACACCGGATTAACTGAGAATTCACAAGTTTTAGAGCTAGAAATAG
pNanog5	GTGGAAAGGACGAAACACCGCGCCAGGAGGGGTGGGTCTAGTTTTAGAGCTAGAAATAG
pCDH1_5	GTGGAAAGGACGAAACACCGGAGACAAGTCGGGGCGGACAGTTTTAGAGCTAGAAATAG
pCDH1_4	GTGGAAAGGACGAAACACCGTCAGAAAGGGCTTTTACACTGTTTTAGAGCTAGAAATAG
pCDH1_3	GTGGAAAGGACGAAACACCGGTCTTAGTGAGCCACCGGCGGTTTTAGAGCTAGAAATAG
pCDH1_2	GTGGAAAGGACGAAACACCGCAGTGGAATCAGAACCGTGCGTTTTAGAGCTAGAAATAG
pCDH1_1	GTGGAAAGGACGAAACACCGAGGGTCACCGCGTCTATGCGGTTTTAGAGCTAGAAATAG
pKLF4_5	GTGGAAAGGACGAAACACCGTCTTCGCGGGCTTCGAACCCGTTTTAGAGCTAGAAATAG
pKLF4_4	GTGGAAAGGACGAAACACCGGTTCGGTCGCTGCGCGACCAGTTTTAGAGCTAGAAATAG
pKLF4_3	GTGGAAAGGACGAAACACCGGCTGCCATAGCAACGATGGAGTTTTAGAGCTAGAAATAG
pKLF4_2	GTGGAAAGGACGAAACACCGTATAAGTAAGGAACGCGCGCGTTTTAGAGCTAGAAATAG
pKLF4_1	GTGGAAAGGACGAAACACCGCGAACGTGTCTGCGGGCGCGGTTTTAGAGCTAGAAATAG
pLIN28_5	GTGGAAAGGACGAAACACCGTCTGATTGGCCAGCGCCGCCGTTTTAGAGCTAGAAATAG
pLIN28_4	GTGGAAAGGACGAAACACCGTAATTATCTGCCCGGGGGGTGTTTTAGAGCTAGAAATAG
pLIN28_3	GTGGAAAGGACGAAACACCGCGGGGTACTCAAGTCTTCTAGTTTTAGAGCTAGAAATAG
pLIN28_2	GTGGAAAGGACGAAACACCGCCCATCTCCAGTTGTGCGTGGTTTTAGAGCTAGAAATAG
pLIN28_1	GTGGAAAGGACGAAACACCGGTGTCAGAGACCGGAGTTGTGTTTTAGAGCTAGAAATAG
pSox2_1	GTGGAAAGGACGAAACACCGTGTAAGGTAAGAGAGGAGAGGTTTTAGAGCTAGAAATAG
pSox2_2	GTGGAAAGGACGAAACACCGTTTACCCACTTCCTTCGAAAGTTTTAGAGCTAGAAATAG
pSox2_3	GTGGAAAGGACGAAACACCGGTGGCTGGCAGGCTGGCTCTGTTTTAGAGCTAGAAATAG
pSox2_4	GTGGAAAGGACGAAACACCGCAAAACCCGGCAGCGAGGCTGTTTTAGAGCTAGAAATAG
pSox2_5	GTGGAAAGGACGAAACACCGAGGAGCCGCCGCGCGCTGATGTTTTAGAGCTAGAAATAG
pFoxA2_1	GTGGAAAGGACGAAACACCGAGTGCCGAGCTGCCCCGAGGGTTTTAGAGCTAGAAATAG
pFoxA2_2	GTGGAAAGGACGAAACACCGCGCGCGGCGCGGGGGCTAGTGTTTTAGAGCTAGAAATAG

pFoxA2_3	GTGGAAAGGACGAAACACCGTGCGGCACTTGTCCGCTCCGGTTTTAGAGCTAGAAATAG
pFoxA2_4	GTGGAAAGGACGAAACACCGTATAGCGCGGCGCGCTGGCGGTTTTAGAGCTAGAAATAG
pFoxA2_5	GTGGAAAGGACGAAACACCGAAATGGGCTGCCCCGGGTCTGTTTTAGAGCTAGAAATAG
pPdx_1	GTGGAAAGGACGAAACACCGGCCCCACGTGGTTCAGCCGGGTTTTAGAGCTAGAAATAG
pPdx_2	GTGGAAAGGACGAAACACCGGCCTGGCTGGCCGCACTAAGGTTTTAGAGCTAGAAATAG
pPdx_3	GTGGAAAGGACGAAACACCGAGCAGGTGCTCGCGGGTACCGTTTTAGAGCTAGAAATAG
pPdx_4	GTGGAAAGGACGAAACACCGGTTTGCTGCACACTCCTGAAGTTTTAGAGCTAGAAATAG
pPdx_5	GTGGAAAGGACGAAACACCGGTTTTCGTGAGCGCCCATTTGTTTTAGAGCTAGAAATAG
pNkx6.1_1	GTGGAAAGGACGAAACACCGGTAGCGCACTTTGAACAGCTGTTTTAGAGCTAGAAATAG
pNkx6.1_2	GTGGAAAGGACGAAACACCGAAACTCTCCGGAGCCAGCCTGTTTTAGAGCTAGAAATAG
pNkx6.1_3	GTGGAAAGGACGAAACACCGAGGACGCCTTGTGCAGCCCGGTTTTAGAGCTAGAAATAG
pNkx6.1_4	GTGGAAAGGACGAAACACCGCCGAATCTCCACTTTGAAGTGTTTTAGAGCTAGAAATAG
pNkx6.1_5	GTGGAAAGGACGAAACACCGGCTCTGCTCTTTCGGTCGCGGTTTTAGAGCTAGAAATAG
gSox17_1	GTGGAAAGGACGAAACACCGGGGCGTGGGCCTAACGACGCGTTTAAGAGCTATGCTGGA
gSox17_2	GTGGAAAGGACGAAACACCGGTGGGGTTGGACTGGGACGTGTTTAAGAGCTATGCTGGA
gSox17_3	GTGGAAAGGACGAAACACCGGCTCCGGCTAGTTTTCCCGGGTTTAAGAGCTATGCTGGA
gSox17_4	GTGGAAAGGACGAAACACCGTCGAGTCTCCCTAACCCCGGGTTTAAGAGCTATGCTGGA
gSox17_5	GTGGAAAGGACGAAACACCGGGGCAAGTACGTCGATTCCAGTTTAAGAGCTATGCTGGA
gGATA4_1	GTGGAAAGGACGAAACACCGACCTCCAAGGAATCCGGGGCGTTTTAGAGCTAGAAATAG
gGATA4_2	GTGGAAAGGACGAAACACCGCTCAACTCTCGATCTTGTGTGTTTTAGAGCTAGAAATAG
gGATA4_3	GTGGAAAGGACGAAACACCGCAGCGAACCCAATCGACCTCGTTTTAGAGCTAGAAATAG
gGATA4_4	GTGGAAAGGACGAAACACCGAATGCCCAAGTGCTACCGCCGTTTTAGAGCTAGAAATAG
gGATA4_5	GTGGAAAGGACGAAACACCGCCTGTGGGAGTCACGTGCAAGTTTTAGAGCTAGAAATAG
tetOp1	GTGGAAAGGACGAAACACCGGTACCTTCTCTATCACTGATGTTTTAGAGCTAGAAATAG
tetOp2	GTGGAAAGGACGAAACACCGGGACTTCTCTATCACTGATAGTTTTAGAGCTAGAAATAG
tetOp3	GTGGAAAGGACGAAACACCGGGGGAGACGTGCGGCCAGCTGTTTTAGAGCTAGAAATAG

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